Claims

1. Microbial host cells which are genetically modified for enhanced synthesis of at least one polyketide, wherein said modification comprises incorporation of at least one expression system for producing a protein that catalyzes the production of starter and/or extender units and/or disabling at least one endogenous pathway for catabolism of starter and/or extender units.

2. A method to produce a polyketide which method comprises culturing the cells of claim 1 under conditions wherein said polyketide is produced.

3. A method to assess the results of a procedure effecting modification of polyketide synthase genes, resulting in a mixture of said modified genes which method comprises

transfecting a culture of cells of claim 1 with said mixture of modified genes, wherein said cells are *E. coli*,

culturing individual colonies of said transformed *E. coli*, and

assessing each colony for polyketide production

4. A method to determine whether a substituted benzoate can prime an adenylation-thiolation (A-T) didomain of a rifamycin synthetase comprising

incubating a substituted benzoate with a *holo* A-T didomain under conditions suitable for priming the A-T didomain; and

measuring the amount or presence of the substituted benzoate that primed the A-T didomain.

5. Procaryotic host cells which do not produce a polyketide in the absence of genetic modification and which are genetically modified for enhanced synthesis of at least one hybrid polyketide, wherein said modification comprises incorporation of at least one expression system comprising an A-T didomain, which incorporates a starter unit that primes an A-T didomain according to the method of claim 4.

6. The procaryotic host cells defined in claim 5 wherein the starter unit is selected from the group consisting of 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, 3-amino-5-hydroxybenzoate, 3-amino-4-hydroxybenzoate, 4-amino-2-hydroxybenzoate, 3-bromobenzoate, 3-chlorobenzoate, 3,5-diaminobenzoate, 3,5-dibromobenzoate, 3,5-dichlorobenzoate, 3,5-difluorobenzoate, 2,3-dihydroxybenzoate, 3,5-dihydroxybenzoate, 3,5-dinitrobenzoate, 3-fluorobenzoate, 2-hydroxybenzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-methoxybenzoate, 3-nitrobenzoate, and 3-sulfobenzoate to make a modified polyketide.

7. The procaryotic host cells defined in claim 5 wherein the starter unit is selected from the group consisting of 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, 3-amino-4-hydroxybenzoate, 4-amino-2-hydroxybenzoate, 3-bromobenzoate, 3-chlorobenzoate, 3,5-diaminobenzoate, 3,5-dibromobenzoate, 3,5-dichlorobenzoate, 3,5-difluorobenzoate, 2,3-dihydroxybenzoate, 3,5-dinitrobenzoate, 3-fluorobenzoate, 2-hydroxybenzoate, 4-hydroxybenzoate, 3-methoxybenzoate, 3-nitrobenzoate, and 3-sulfobenzoate to make a modified polyketide.

8. A hybrid polyketide in which a starter unit is incorporated therein which starter unit primes an A-T didomain according to the method of claim 4.

9. The hybrid polyketide defined in claim 8 where the starter unit is selected from the group consisting of 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, 3-amino-5-hydroxybenzoate, 3-amino-4-hydroxybenzoate, 4-amino-2-hydroxybenzoate, 3-bromobenzoate, 3-chlorobenzoate, 3,5-diaminobenzoate, 3,5-dibromobenzoate, 3,5-dichlorobenzoate, 3,5-difluorobenzoate, 2,3-dihydroxybenzoate, 3,5-dihydroxybenzoate, 3,5-dinitrobenzoate, 3-fluorobenzoate, 2-hydroxybenzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-methoxybenzoate, 3-nitrobenzoate, and 3-sulfobenzoate.

10. The hybrid polyketide defined in claim 9 where the starter unit is selected from the group consisting of 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, 3-amino-4-hydroxybenzoate, 4-amino-2-hydroxybenzoate, 3-bromobenzoate, 3-chlorobenzoate, 3,5-diaminobenzoate, 3,5-dibromobenzoate, 3,5-dichlorobenzoate, 3,5-difluorobenzoate,

2,3-dihydroxybenzoate, 3,5-dinitrobenzoate, 3-fluorobenzoate, 2-hydroxybenzoate, 4-hydroxybenzoate, 3-methoxybenzoate, 3-nitrobenzoate, and 3-sulfobenzoate.

11. A method to produce a polyketide which method comprises culturing the cells of claim 5 under conditions wherein said polyketide is produced.

12. The cells of claim 5 which are of the genus *Escherichia*, *Streptomyces*, *Bacillus*, *Pseudomonas*, or *Flavobacterium*.

13. The cells of claim 12 which are *E. coli*.

14. The cells of claim 5 wherein said cells produce a complete polyketide derived from rifamycin, rapamycin, FK506, ansatrienin, FK520, microcystin, pimaricin, erythromycin, oleandomycin, megalomycin, picromycin, spinosad, avermectin, tylosin or epothilone.

15. The cells of claim 14 which produce a modified rifamycin.

16. The cells of claim 14 which produce a 6-dEB analog.

17. The cells of claim 5, wherein said genetic modification further comprises incorporation of at least one expression system for a polyketide synthase protein.

18. The cells of claim 5 wherein said genetic modification comprises incorporation of at least one expression system for a phosphopantetheinyl transferase.

19. A method to enhance the production of at least one hybrid polyketide in a microbial host which method comprises providing said host with an expression system for producing a protein that incorporates an exogenous starter unit that primes an A-T didomain according to the method of claim 4.

20. A method to enhance production of a secondary metabolite in a microbial host which method comprises inducing the production of said secondary metabolite after the microbial cells have reached a high cell density in the culture.

21. The method of claim 20 wherein the secondary metabolite is a polyketide.

22. The method of claim 20 wherein said inducing is by supplying a compound which activates an inducible promoter.

23. A method to enhance the production of a secondary metabolite in a microbial host which method comprises maintaining the nutrient level fed to said microbe at a relatively constant level during the production of said secondary metabolite.

24. The method of claim 23 wherein said secondary metabolite is a polyketide.

25. A method to enhance the production of a polyketide in a microbe which method comprises modifying said microbe to contain an expression system for a thioesterase II (TEII).